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COMMUNICATIONS

Published on Web 09/07/2006

Chemically Selective Sensing through Layer-by-Layer Incorporation of Biorecognition into Thin Film Substrates for Surface-Enhanced Resonance Raman Scattering

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Owing primarily to their surface plasmon resonances, noble metal nanoparticles possess some of the most interesting properties of all nanoscale materials.[1] These resonances are highly sensitive to particle size, shape, and environment, making them attractive for use in a wide variety of applications.[2] Among the most exciting of these, are those where metallic nanoparticles are incorporated into systems that exploit strong biospecific interactions,[3] including, among others, those of antigen/antibody,[4] protein/small molecule,[5] and nucleic acid systems.[6] Particularly promising among possible strategies for coupling the unique optical properties of metallic nanoparticles with the functionality of chemically selective materials is the utilization of the layer-by-layer (LbL) technique for thin film fabrication.

The layer-by-layer technique, pioneered by Decher,[7] has been shown to be an extremely successful method for creating nanocomposite films with tailored properties and functionality, through consecutive, alternating adsorption steps of a variety of materials including polymers,[8] proteins,[9] quantum dots,[10] and metallic nanoparticles.[11] The presence of optically active nanoparticles within LbL architectures allows adsorption events to be observed by several methods including localized surface plasmon resonance (LSPR),[12] surface-enhanced fluorescence (SEF),[13] and surface-enhanced resonance Raman scattering (SERRS).[14] The SERRS effect increases latent Raman scattering signals by several orders of magnitude, through molecular resonance and electromagnetic (EM) enhancement mechanisms, and is now routinely employed for trace vibrational analysis down to the single molecule level.

Here, we demonstrate the fabrication, characterization, and utilization, as chemically selective SERRS substrates, of LbL films composed of the glycoprotein avidin and colloidal Ag nanoparticles. The strong, biospecific interaction between avidin and the small molecule biotin, one of the strongest known to exist in nature, is exploited to preferentially capture biotinylated species from solution (Figure 1), leading to effective "concentration enhancement" of SERRS signals.

The colloidal Ag nanoparticles employed here are negatively charged, with a measured zeta potential of ca. −50 mV at neutral pH,[15] while avidin is strongly cationic, having an isoelectric point of 10.5, thus making LbL film construction through electrostatic interactions possible. To produce these films, clean, silanized glass substrates were immersed alternately, for 30 min, with intervening water rinsing steps, into solutions of citrate reduced Ag colloids (2x diluted)[16] and avidin (50 μg/mL in 10mM phosphate buffered saline, PBS, at pH 7.5), until 14 bilayers were deposited (film growth was studied fully and will be presented elsewhere[17]).

Once fabricated, these 14 bilayer films were characterized by UV−visible absorption and atomic force microscopy (AFM) measurements (Figure 2). They were found to exhibit three surface plasmon absorption bands at ca. 383, 430, and 700 nm, corresponding to quadropole, dipole, and particle aggregate absorption modes, respectively. They were also found, by noncontact tapping mode AFM imaging, to be composed of individual Ag particles ranging between 30 and 70 nm in diameter and larger particle aggregates ranging between 150 and 500 nm in diameter. These results, importantly, confirm the presence of interacting nanoparticle clusters that are well-known to be the source of the strongest EM enhancements of Raman and resonance Raman scattering.

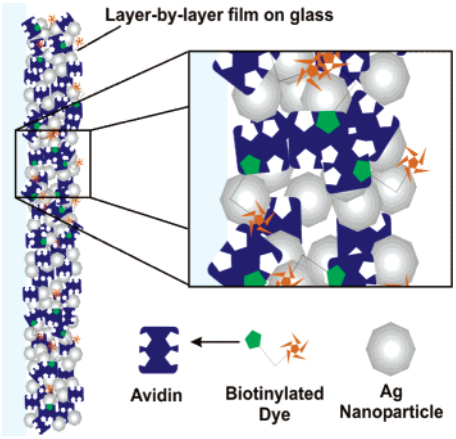

Figure 1. Schematic depiction of avidin/Ag nanoparticle layer-by-layer film and its selective adsorption of biotinylated species from solution.

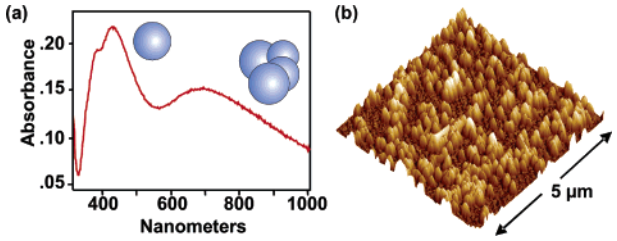

Figure 2. (a) Surface plasmon and (b) AFM image of avidin/Ag nanoparticle layer-by-layer film.

The ability of these avidin/Ag nanoparticle LbL films to act as chemically selective SERRS substrates was tested and the results are shown in Figure 3. An avidin/Ag nanoparticle film was immersed into a 10^{-4} M solution of a commercially available, biotinylated fluorescein dye, biotin-4-fluorescein (B4F) for 30 min, while another was immersed into a solution of nontagged fluorescein, at the same concentration, for the same period of time. Both samples were removed, rinsed with distilled water, and dried before SERRS measurements were made using 514 nm laser excitation. The spectra obtained from these samples were found to be essentially the same; however, the biotinylated sample was found to exhibit absolute intensities that were, on average, 10^2 greater

10.1021/ja0644327 CCC: $33.50

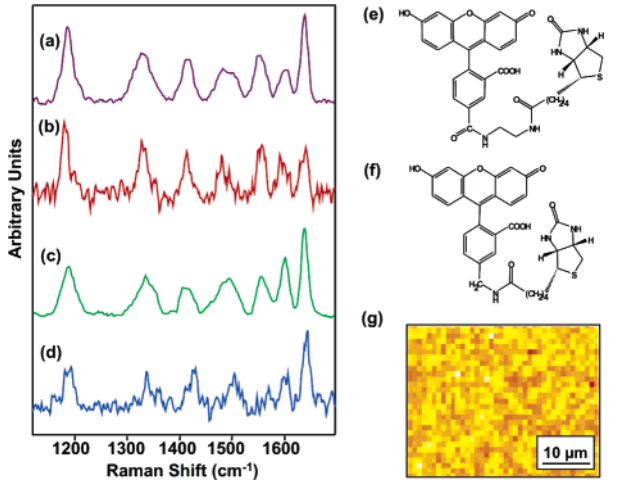

Figure 3. SERRS spectra, recorded using 514.5 nm excitation, from avidin/Ag nanoparticle layer-by-layer film dipped into (a) 10^{-4} and (b) 10^{-7} M biotin-4-fluorescein and (c) 10^{-4} and (d) 10^{-7} M biotinylated 5-(aminomethyl)fluorescein solutions. Shown, in parts e and f, respectively, are the structures of these biotinylated species. In part g, a 2D spatial map of SERRS intensities for biotin-4-fluorecein, showing highly uniform response, is shown.

than those measured for its nontagged counterpart. It was also found that the nontagged fluorescein could not be observed at all spots and was near its detection limit, while biotin-4-fluorescein could be readily observed at all spots with strong intensity. The uniformity of this response is demonstrated in the 2D spatial SERRS mapping results of Figure 3g. Moreover, B4F was found to be detectable from substrates dipped, using identical procedures, into solutions with concentrations as low as 10^{-7} M, whereas nontagged fluorescein was undetectable when extracted from solutions with concentrations below 10^{-5} M. In both cases, SERRS spectra result from the same resonant, central chromophore, with an unchanged Raman cross section. Therefore the 100 fold increase in intensity, and related 100 fold increase in detection sensitivity, associated with biotinylation, can be attributed to a concentration enhancement that arises specifically as a result of the strong, biospecific interaction between the avidin in the LbL film and the biotin tag.

The concentration enhancement and improved detection limits reported here for presynthesized biotinylated species (which are numerous) are clearly significant; however, it is also interesting to pursue similar results for analytes biotinylated in situ in aqueous solution. This approach further broadens the possible uses for this substrate, as there are many well-established biotinylation procedures available in the literature. Here, as a proof of concept, a water soluble biotinylation reagent, biotin 3-sulfo-*N*-hydroxysuccinimide ester was reacted in situ with 5-(aminomethyl)fluorescein (5-AF) in pH 7.5 PBS solution. The two reagents were combined in a 1:1 molar ratio, at room temperature, to produce a solution 10^{-4} M in each. The reaction was allowed 30 min for completion before LbL film substrates were immersed into it, and a solution of nontagged 5-AF, for 30 min. Upon removal, the films were rinsed and dried, and SERRS spectra were recorded. It was found that in situ biotinylation yielded similar 10^2 improvements in signal intensity as a result of concentration enhancement. Moreover, it was found that biotinylated 5-AF was also detectable down to 10^{-7} M, with a corresponding 10^2 advantage in detection limits over its nontagged counterpart. Finally, to ensure that observed enhancements were indeed the result of preferential capture of biotinylated species, films were immersed into concentrated solutions of biotin before immersion into solutions of the two biotinylated analytes. In both cases, saturation of the surface with biotin was in fact found to greatly reduce SERRS intensities, thus confirming the importance of biorecognition in this work.

In summary, a versatile new system for coupling the functionality of avidin with the unique optical properties of Ag nanoparticles has been developed using the LbL technique. The fabrication, characterization, and application of these nanocomposite films toward SERRS studies, has been demonstrated for two biotinylated species, with preferential adsorption yielding concentration enhancements and improved detection sensitivities of ca. 10^2 for each. It is expected that the concepts reported here can be extended toward the fabrication of a wide variety of "smart" SERS/SERRS substrates incorporating various biorecognition systems into LbL architectures.

Acknowledgment. Financial assistance from NSERC of Canada is gratefully acknowledged.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) El-Sayed, M. A. *Acc. Chem. Res.* **2001**, *34*, 257−264.
(2) Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. *J. Phys. Chem. B* **2003**, *107*, 668−677.
(3) Penn, S. G.; He, L.; Natan, M. J. *Curr. Opin. Chem. Biol.* **2003**, *7*, 609−615.
(4) Englebienne, P. *Analyst* **1998**, *123*, 1599−1603.
(5) Kim, N. H.; Lee, S. J.; Kim, K. *Chem. Commun.* **2003**, *6*, 724−725.
(6) Cao, Y. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536−1540.
(7) Decher, G. *Science* **1997**, *277*, 1232−1237.
(8) dos Santos, D. S., Jr.; Riul, A., Jr.; Malmegrim, R. R.; Fonseca, F. J.; Oliveira, O. N., Jr.; Mattoso, L. H. C. *Macromol. Biosci.* **2003**, *3*, 591−595.
(9) Anzai, J.; Kobayashi, Y.; Nakamura, N.; Nishimura, M.; Hoshi, T. *Langmuir* **1999**, *15*, 221−226.
(10) Zhang, H.; Zhou, Z.; Liu, K.; Wang, R.; Yang, B. *J. Mater. Chem.* **2003**, *13*, 1356−1361.
(11) Goulet, P. J. G.; Dos Santos, D. S., Jr.; Alvarez-Puebla, R. A.; Oliveira, O. N., Jr.; Aroca, R. F. *Langmuir* **2005**, *21*, 5576−5581.
(12) Haes, A. J.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2002**, *124*, 10596−10604.
(13) Maliwal, B. P.; Malicka, J.; Gryczynski, I.; Gryczynski, Z.; Lakowicz, J. R. *Biopolymers* **2003**, *70*, 585−594.
(14) Ni, F.; Cotton, T. M. *J. Raman Spectrosc.* **1988**, *19*, 429−438.
(15) Alvarez-Puebla, R. A.; Arceo, E.; Goulet, P. J. G.; Garrido, J. J.; Aroca, R. F. *J. Phys. Chem. B* **2005**, *109*, 3787−3792.
(16) Lee, P. C.; Meisel, D. *J. Phys. Chem.* **1982**, *86*, 3391−3395.
(17) Goulet, P. J. G.; Pieczonka, N. P. W.; Aroca, R. F. In *New Approaches in Biomedical Spectroscopy*; Kneipp, K., Aroca, R. F., Kneipp, H., Wentrup-Byrne, E., Eds.; American Chemical Society: Washington, DC, in press.

JA0644327